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## Bioresorbable Bioactive Bone Chips: A New 3D Scaffold for Tissue Engineering in Reconstructive Maxillo-Facial Surgery

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### Abstract

Scaffolds for bone tissue engineering (t.e.) should ensure both mechanical stability and adequate strength. Moreover, their intimate structure should have an interconnected porous network for cell migration and proliferation, while also providing specific signals for bone regeneration. In this framework of know requirements, our approach was focused in finding a composite solution, bearing cues from both mineral components and polymeric ones. Indeed, here we present the first results of

a new composite 3D scaffold thought for bone t.e., particularly for maxillo-facial surgery, using a novel concept based on biomaterial assembly.

This novel scaffold has a composite structure based on a bovine derived bone matrix specifically reinforced with biodegradable polymers and bioactive agents. The bovine derived matrix allows to maintain an adequate 3D-structure and porosity, the biopolymers permit to achieve good mechanical properties while bioactive agents promote cell adhesion

*and proliferation. Scaffolds are produced according to GMP standards applying components which are all approved for human use.*

*Microstructure analysis confirmed a morphology similar to human cortical bone, while handling and mechanical assessment confirmed adequate strength and resistance.*

*Moreover, citocompatibility and cell viability confirmed the ability of this matrix to support and to promote cells proliferation.*

### **Keywords**

Bone scaffold, tissue engineering, polymeric reinforcement, reconstructive surgery, composite technology.

### **1 Introduction**

Scaffolds for bone tissue engineering (t.e.) should ensure both mechanical stability and adequate strength. Moreover, their microstructure should have an interconnected porous network for cell migration and proliferation, while also providing specific signals for bone regeneration [1]. These specifications have been identified as stringent needs ever since early maxillo-facial surgery in the 80's [2]. Furthermore, connective tissue adhesion should be promoted too. In this framework, biopolymers [3], bioglass and bioceramics [4] are often considered to satisfy these requirements, at least in part [5]. Our approach was focused in finding a composite solution, bearing cues from both mineral components and polymeric ones. Indeed, here we present the first results of a new composite 3D scaffold thought for bone t.e., particularly for maxillo-facial surgery, using a novel concept based on biomaterial assembly [6], thus avoiding drawbacks of interfacial bonding.

### **2 Materials and Methods**

This novel scaffold has a composite structure based on a bovine derived bone matrix specifically reinforced with biodegradable polymers and bioactive agents. The bovine derived matrix allows to maintain an adequate 3D-structure and porosity, the biopolymers permit to achieve good mechanical properties while bioactive agents promote cell adhesion and proliferation. Scaffolds are produced according to GMP standards (at the Micro-Sphere S/A facility) applying components which are all approved for human use. Both process and product are proprietary [6].

Microstructure was evaluated by E/SEM imaging (Evo 50EP, Zeiss-Cambridge Instruments). Biomechanical behaviour was assessed by monoaxial compression tests (MTS 858 MiniBionix), performed at constant compression speed of 1mm/60sec, comparing maximum linear stress and Young's module of the new scaffold with those of the pure bovine derived matrix.

Handling tests were performed by surgeons to assess applicability in oral surgery.

Citocompatibility and cell viability were assessed by means of *in vitro* tests, performed with bovine chondrocytes and MG63 line cells (human osteosarcoma), aiming at evaluating the ability to support and to promote cells proliferation within the matrix.

### **3 Results**

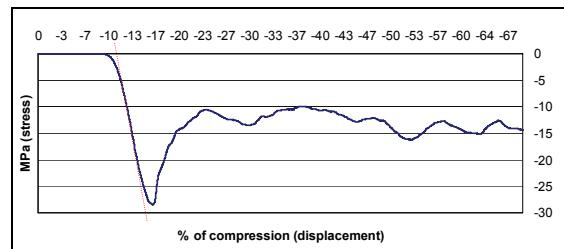
This novel composite 3D scaffold resulted not only feasible for oral surgery, being both easy to shape and resistant to screws and fixation manoeuvres (as depicted in *Fig 1*), but also better performing than nowadays available pure bovine derived matrix. Mechanical data indeed show that the polymeric reinforcement process improves by

a factor 3 the maximum stress and the Young's module, both being evaluated along the linear field. Specifically, as depicted in *Fig 2*, the scaffold compression behaviour is that typical of an open cellular structure where a first pseudo-linear and pseudo-elastic behaviour, due to structural resistance, is then followed by oscillating behaviour due to progressive breakage of structure and consequent compacting of matrix. Stress, thus, appears to remain high but this effect is just due to pure compacting resistance, as structural integrity is definitely lost after the linear tract.

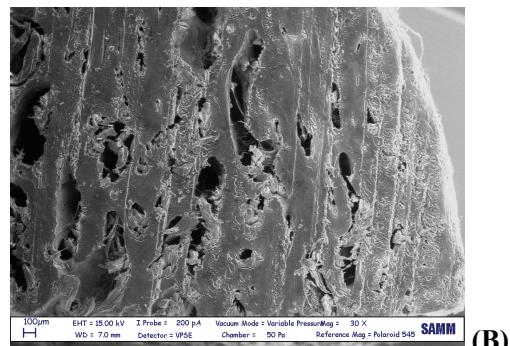
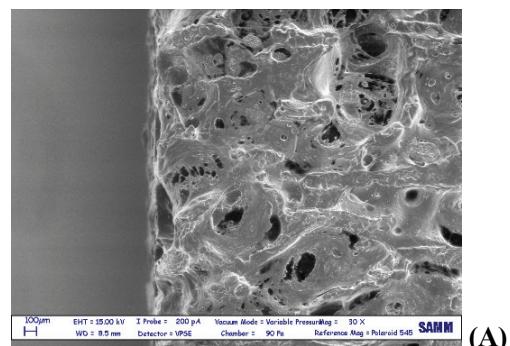


*Figure 1.* bone chip (about 1c.c.) appears easy to be handled and shaped by surgeons to be adapted to local defects and needs.

Moreover, SEM imaging confirmed that its microscopic structure resembles that of human cortical bone (as evident from *Fig 3*): i.e. an open porous cellular matrix. Cell proliferation, assessed by Alamar Blue<sup>TM</sup> test, and viability resulted both positive and fully comparable in terms of values referred to controls. Further confirmations came from morphological analysis performed via SEM imaging.



*Figure 2.* the scaffold compression test plot show a typical behaviour of an open cellular structure where a first pseudo-linear and pseudo-elastic field (red dots), due to structural resistance, is followed by oscillating behaviour, due to progressive breakage of structure and consequent compacting of matrix.



*Figure 3.* Microscopic structure of new composite scaffold (a) evidences strong affinities with human cortical bone (b), particularly related to porosity which appears completely comparable in terms of size and cells seeding spaces to human cortical bone (b).

#### 4 Conclusions

Data collected represent a positive proof of concept for the developed process and the application of this new type of matrix as scaffold for bone tissue regeneration. Morphological analysis of structure confirmed the presence of a well diffused cortical bone human-like porosity. Preliminary mechanical investigation showed easy shaping by common surgical instruments in order to replicate and thus replace bone defects and a relevant improved resistance to compression with respect to nowadays available solutions. Furthermore, biological investigations showed scaffolds to be promising substrates for cell adhesion and growth, being strongly biocompatible and enhancing cell viability.

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